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=> d que stat l10
L1             7 SEA FILE=REGISTRY ABB=ON  (IRON OR CALCIUM OR ALUMINUM OR
                 NICKEL OR ZINC OR COBALT OR COPPER)/CN
L2        111827 SEA FILE=HCAPLUS ABB=ON  (?PEPTID? OR ?PROTEIN?)(4A)(?PURIF?
                 OR ?WASH? OR ?EXTRACT? OR ?ELUT?)
L3          3656 SEA FILE=HCAPLUS ABB=ON  L2 AND (?CHELAT? OR ?RESIN?)
L4          1288 SEA FILE=HCAPLUS ABB=ON  L3 AND (L1 OR ?IRON? OR FE OR
                 ?CALCIUM? OR CA OR ?ALUMINUM? OR AL OR ?NICKEL? OR NI OR
                 ?ZINC? OR ZN OR ?COBALT? OR CO OR ?COPPER? OR CU)
L5           229 SEA FILE=HCAPLUS ABB=ON  L4 AND (?FUSE? OR ?FUSION?)
L6           177 SEA FILE=HCAPLUS ABB=ON  L5 AND (?CHROMATOG? OR ?BUFFER? OR
                 ?COLUMN?)
L7             4 SEA FILE=HCAPLUS ABB=ON  L6 AND (?HARD? OR ?INTERMED?)
L8             6 SEA FILE=HCAPLUS ABB=ON  L6 AND KIT?
L9             9 SEA FILE=HCAPLUS ABB=ON  L7 OR L8
L10            9 SEA FILE=HCAPLUS ABB=ON  L7 OR L8 OR L9

=> d ibib abs l10 1-9

L10  ANSWER 1 OF 9  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2005:34869  HCAPLUS
TITLE:                    Methods and compositions for enhanced protein
                          expression and purification
INVENTOR(S):              Butt, Tauseef R.; Malakhov, Michael P.; Malakhova,
                          Oxana A.
PATENT ASSIGNEE(S):       Lifesensors, Inc., USA
SOURCE:                   PCT Int. Appl., 107 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
     WO 2005003313         A2    20050113    WO 2004-US20778         20040628
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
             AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
             EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,
             SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
             SN, TD, TG
PRIORITY APPLN. INFO.:                        US 2003-482817P     P  20030626
AB   Methods for enhancing expression levels and secretion of heterologous
     fusion proteins in a host cell are disclosed.  The method
     comprises: mol. cloning of COOH-terminal of a Ubl mol. selected from the
     group consisting of SUMO, UB, RUB, HIB, APG8, APG12, URM1, Ubi-L, FAT10
     and ISG15 to a nucleic acid sequence encoding the protein of interest.
     The invention further relates to the compns. and purification of the
     protein.

L10  ANSWER 2 OF 9  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2004:905784  HCAPLUS
DOCUMENT NUMBER:          141:376819
TITLE:                    Affinity purification system using troponin molecules
```

as affinity ligands
INVENTOR(S): Moeckli, Randolph A.; Chadwick, Christopher C.
PATENT ASSIGNEE(S): Aristex, USA
SOURCE: PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004092214	A2	20041028	WO 2004-US10887	20040407
WO 2004092214	A3	20041118		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-462483P P 20030410

AB This invention pertains to the use of a novel set of tags for the immobilization and/or **purification** of **proteins** or other biol. or organic mols. The invention provides troponin C, troponin C binding peptide, and troponin I, or active fragments or analogs thereof as convenient tags and affinity ligands for immobilizing, attaching, or **purifying proteins** or other mols. Methods for producing troponin-tagged mols., such as recombinant **fusion** proteins, are described. Methods for preparing a troponin affinity matrix that is capable of specifically binding its cognate ligand in the presence of **calcium**, and methods for using such a matrix to purify troponin-tagged mols. are also described.

L10 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:759732 HCAPLUS
DOCUMENT NUMBER: 141:273989
TITLE: **Purification** of **fusion proteins** using immobilized bi-metal affinity **chromatography**
INVENTOR(S): Tchaga, Grigoriy S.; Jokhadze, George G.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp., Cont.-in-part of U.S. Pat. Appl. 2002 164,718.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004180415	A1	20040916	US 2004-762588	20040121
US 2002164718	A1	20021107	US 2001-858332	20010515

PRIORITY APPLN. INFO.: US 2001-858332 A2 20010515
US 2003-441804P P 20030121

mine

US 1998-101867P P 19980925
US 1999-404017 B2 19990923

AB The present invention relates to IMAC (Immobilized Metal Affinity **Chromatog.**). The present invention provides methods of **purifying proteins** that include a metal ion affinity peptide. The methods generally involve contacting a **fusion** protein that includes a metal ion affinity peptide with at least two different metal ion **chelating resins.** In certain representative embodiments, the methods include contacting a **fusion** protein with a first metal ion **chelate resin** having a first immobilized metal ion; **eluting** any bound **protein** from the first metal ion **chelate resin**, to produce an eluate; contacting the eluate with a second metal ion **chelate resin** having a second immobilized metal ion; and **eluting** any bound **protein** from the second metal ion **chelate resin.** Also provided are **kits** for use in practicing the subject methods. An illustrative purification protocol for Bi-MAC (Bi-Metal Affinity **Chromatog.**) is shown. The subject methods find use in a variety of **protein purification** applications.

L10 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:790100 HCAPLUS
DOCUMENT NUMBER: 140:176405
TITLE: Molecular cloning, overexpression in Escherichia coli, and purification of 6+ his-tagged C-terminal domain of Clostridium difficile toxins A and B
AUTHOR(S): Letourneur, Odile; Ottone, Sophie; Delauzun, Vincent; Bastide, Marie-Claire; Foussadier, Agnes
CORPORATE SOURCE: BioMerieux, Chemin de l'Orme, Marcy l'Etoile, 69280, Fr.
SOURCE: Protein Expression and Purification (2003), 31(2), 276-285
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Genomic DNA from ribotype-01 and -17 Clostridium difficile strains was used for amplification of the sequences encoding the carboxy-terminal domain of toxins A (TcdA) and B (TcdB). The deduced C-terminal TcdB ribotype-01 and -17 domains share 99.5% amino acid sequence identity while TcdA ribotype-17 comprises a 607 amino acid deletion compared to TcdA-01. When compared to previously sequenced C. difficile toxins, 99.3% amino acid identity was found between TcdA-01 and TcdA from strain VPI10643 and 98.8% identity between TcdA-17 and TcdA from strain F-1470. The obtained sequences were **fused** in 3' to a sequence encoding a hexahistidine tag and cloned into an Escherichia coli expression vector. The recombinant proteins were expressed in E. coli and purified using single-step metal-**chelate chromatog.** The recombinant carboxy-terminal domain of TcdA-01 was purified from the soluble E. coli lysate fraction whereas TcdA-17 and TcdB-17 carboxy-terminal domains were purified from inclusion bodies. At least 40 mg of each **protein** was **purified** per L of bacterial culture. The recombinant toxin domains were detected specifically by Western blot and ELISA with antibodies against native C. difficile toxins. This study demonstrated that the carboxy-terminal domains of TcdA and TcdB can be produced using an E. coli expression system and easily purified. These recombinant, stable, and non-toxic proteins provide a convenient source for use in the diagnosis of C. difficile infections, instead of native toxins, as controls and calibrators in immunoassay **kits** and to obtain

specific monoclonal antibodies.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:78239 HCAPLUS
DOCUMENT NUMBER: 136:133614
TITLE: purification and application of IgY (IgY Fc) from egg yolk of immunized duck
INVENTOR(S): Chiu, Yi-neng
PATENT ASSIGNEE(S): Kute Bioscience and Technology Y. K., Taiwan
SOURCE: Jpn. Kokai Tokkyo Koho, 15 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002030100	A2	20020129	JP 2000-200544	20000703
JP 3610551	B2	20050112		

PRIORITY APPLN. INFO.: JP 2000-200544 20000703
AB A method is provided to purify IgY from egg yolk of duck immunized with antigens. In order to avoid the immunogenicity and unnecessary complement activation, it is an advanced development to delete or limit the responsible Fc fragment of the antibody. The antigen-specific IgY (IgY Fc) purified from egg yolk by using immuno-affinity method under conditions such as PH 4-7 and lower than 50 mM of ion concentration provides the possible application in therapy and diagnosis of diseases.

L10 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:508761 HCAPLUS
DOCUMENT NUMBER: 131:254057
TITLE: Selective adsorption of poly-His tagged glutaryl acylase on tailor-made metal **chelate** supports
AUTHOR(S): Armisen, Pilar; Mateo, Cesar; Cortes, Estrella; Barredo, Jose L.; Salto, Francisco; Diez, Bruno; Rodes, Lorenzo; Garcia, Jose L.; Fernandez-Lafuente, Roberto; Guisan, Jose M.
CORPORATE SOURCE: Hispanagar S.A., Burgos, Spain
SOURCE: Journal of Chromatography, A (1999), 848(1 + 2), 61-70
CODEN: JCRAEY; ISSN: 0021-9673
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A poly-His tag was **fused** in the glutaryl acylase (GA) from Acinetobacter sp. strain YS114 cloned in E. coli yielding a fully active enzyme. Biochem. analyses showed that the tag did not alter the maturation of the chimeric GA (poly-His GA) that undergoes a complex post-translational processing from an inactive monomeric precursor to the active heterodimeric enzyme. This enzyme has been used as a model to develop a novel and very simple procedure for one-step **purification** of poly-His **proteins** via immobilized metal-ion affinity **chromatog.** on tailor-made supports. It was intended to improve the selectivity of adsorption of the target protein on tailor-made **chelate** supports instead of performing a selective desorption. The rate and extent of the adsorption of **proteins** from a crude

extract from E. coli and of pure poly-His tagged GA on different metal **chelate** supports was studied. Up to 90% of proteins from E. coli were adsorbed on com. **chelate** supports having a high d. of ligands attached to the support through long spacer arms, while this adsorption becomes almost negligible when using low ligand densities, short spacer arms and Zn2+ or Co2+ as cations. On the contrary, poly-His GA adsorbs strongly enough on all supports. A strong affinity interaction between the poly-His tail and a single **chelate** moiety seems to be the responsible for the adsorption of poly-His GA. By contrast, multipoint weak interactions involving a number of **chelate** moieties seem to be mainly responsible for adsorption of natural proteins. By using tailor-made affinity supports, a very simple procedure for one-step purification of GA with minimal adsorption of host proteins could be performed. Up to 20 mg of GA were adsorbed on each ml of **chelate** support while most of accompanying proteins were **hardly** adsorbed on such supports. Following few washing steps, the target enzyme was finally recovered (80% yield) by elution with 50 mM imidazole with a very high increment of specific activity (up to a 120 purification factor).

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:151235 HCAPLUS
DOCUMENT NUMBER: 128:177564
TITLE: Recombinant myeloperoxidase (MPO) fragment and methods for assaying MPO-specific anti-neutrophile cytoplasmic autoantibody (MPO-ANCA) reaction site and determining MPO-ANCA by using the fragment
INVENTOR(S): Suzuki, Kazuo; Tanokura, Masaru
PATENT ASSIGNEE(S): Teikoku Hormone Mfg. Co., Ltd., Japan; Suzuki, Kazuo; Tanokura, Masaru
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9807848	A1	19980226	WO 1997-JP2910		19970821
W: AU, CA, CN, JP, KR, US					
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
AU 9738677	A1	19980306	AU 1997-38677		19970821
PRIORITY APPLN. INFO.:			JP 1996-219855	A	19960821
			WO 1997-JP2910	W	19970821

AB Disclosed are a recombinant MPO fragment reacting with myeloperoxidase-specific anti-neutrophile cytoplasmic antibody (MPO-ANCA); a process for producing the fragment by expression of the encoding cDNA in Escherichia coli ; a method for assaying the MPO-ANCA reaction site and its amount in a specimen by using the fragment; and reagent **kits** for the assay which consists of a panel set of the fragment and labeled anti-human IgG. PCR amplification to obtain the cDNA encoding 16 human MPO fragments, preparation of MPO fragments **fused** with 6 Histidines in transgenic Escherichia coli, and **purification** of the **fusion protein** by using **Ni**-NTA (**Nickel** -nitrilotriacetate) **resin column** were shown. The MPO fragments are useful in diagnosis of autoimmune diseases.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:104290 HCAPLUS
DOCUMENT NUMBER: 126:195152
TITLE: Naloxone-Sensitive, haloperidol-sensitive, [3H](+)SKF-10047-binding **protein** partially **purified** from rat liver and rat brain membranes: An opioid/Sigma receptor?
AUTHOR(S): Tsao, Li-I.; Su, Tsung-Ping
CORPORATE SOURCE: Molecular Neuropsychiatry Section, Division of Intramural Research, National Institute on Drug Abuse/NIH, Baltimore, MD, USA
SOURCE: Synapse (New York) (1997), 25(2), 117-124
CODEN: SYNAET; ISSN: 0887-4476
PUBLISHER: Wiley-Liss
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A naloxone-sensitive, haloperidol-sensitive, [3H](+)SKF-10047-binding **protein** was partially **purified** from rat liver and rat brain membranes in an affinity **chromatog.** originally designed to purify sigma receptors. Detergent-solubilized exts. from membranes were adsorbed to Sephadex G-25 **resin** containing an affinity ligand for sigma receptors: N-(2-[3,4-dichlorophenyl]ethyl)-N-(6-aminohexyl)-(2-[1-pyrrolidinyl])ethylamine (DAPE). After eluting the **resin** with haloperidol, a protein that bound [3](+)SKF-10047 was detected in the eluates. However, the protein was not the sigma receptor. [3H](+)SKF-10047 binding to the protein was inhibited by the following compds. in the order of decreasing potency: (+)pentazocine > (-)pentazocine > (±)cyclazocine > (-)morphine > (-)naloxone > haloperidol > (+)SKF-10047 > DADLE > (-)SKF-10047. Further, the prototypic sigma receptor ligands, such as 1,3-di-o-tolylguanidine (DTG), (+)3-PPP, and progesterone, bound poorly to the protein. Tryptic digestion and heat treatment of the affinity-**purified protein** abolished radioligand binding. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) of the partially-**purified protein** from the liver revealed a major **diffuse** band with a mol. mass of 31 kDa, a polypeptide of 65 kDa, and another polypeptide of > 97 kDa. This study demonstrates the existence of a novel protein in the rat liver and rat brain which binds opioids, benzomorphans, and haloperidol with nanomolar affinity. The protein resembles the opioid/sigma receptor originally proposed by Martin et **al.** [(1976): J. Pharmacol. Exp. Ther., 197:517-532.]. A high degree of **purification** of this **protein** has been achieved in the present study.

L10 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:706909 HCAPLUS
DOCUMENT NUMBER: 126:15280
TITLE: A multifunctional vector system for heterologous expression of proteins in Escherichia coli. Expression of native and hexahistidyl **fusion proteins**, rapid **purification** of the **fusion proteins**, and removal of **fusion** peptide by Kex2 protease
AUTHOR(S): Ghosh, Smita; Lowenstein, John M.
CORPORATE SOURCE: Biochemistry Department, Brandeis University, Waltham, MA, 02254, USA
SOURCE: Gene (1996), 176(1/2), 249-255
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier

DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vectors have been constructed for the general purpose of expressing foreign proteins in E. coli. These vectors allow the production in high yield of either native proteins or of **fusion** proteins which contain, at their amino terminus, the peptide Met Gly His6 Ser Gly Leu Phe Lys Arg/, where Leu Phe Lys Arg/ is the recognition site for Kex2 protease which cleaves at the site indicated by /. The His6 sequence is used as a ligand for the one-step affinity **purification** of the expressed **proteins** on **columns** containing **Ni** or **Zn** ions **chelated** to iminodiacetic acid-agarose. After affinity **chromatog.**, the **purification peptide** is cleaved off with Kex2 protease from Saccharomyces cerevisiae. The vectors also allow site-directed mutagenesis and sequencing of the cloned gene to be expressed without any **intermediate** subcloning. For practical examples of over-expression, affinity purification, and removal of the **purification peptide**, the authors chose a high-mol.-weight protein, phospholipase Cγ1 (PLCγ1, Mr 148 000) and a low-mol.-weight protein, Hit-1 (Mr 16 000). Both were obtained pure and in high yield. PLCγ1 was fully active; the function of Hit-1 is not known. A set of companion vectors for **co**-expression of addnl. proteins has also been developed. These allow expression of proteins which enhance the production of activity of the protein of primary interest and of proteins which exhibit trans-interactions.

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=> d que stat l12
L1             7 SEA FILE=REGISTRY ABB=ON  (IRON OR CALCIUM OR ALUMINUM OR
                   NICKEL OR ZINC OR COBALT OR COPPER)/CN
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                   OR ?WASH? OR ?EXTRACT? OR ?ELUT?)
L3          3656 SEA FILE=HCAPLUS ABB=ON  L2 AND (?CHELAT? OR ?RESIN?)
L4          1288 SEA FILE=HCAPLUS ABB=ON  L3 AND (L1 OR ?IRON? OR FE OR
                   ?CALCIUM? OR CA OR ?ALUMINUM? OR AL OR ?NICKEL? OR NI OR
                   ?ZINC? OR ZN OR ?COBALT? OR CO OR ?COPPER? OR CU)
L5           229 SEA FILE=HCAPLUS ABB=ON  L4 AND (?FUSE? OR ?FUSION?)
L6           177 SEA FILE=HCAPLUS ABB=ON  L5 AND (?CHROMATOG? OR ?BUFFER? OR
                   ?COLUMN?)
L7             4 SEA FILE=HCAPLUS ABB=ON  L6 AND (?HARD? OR ?INTERMED?)
L8             6 SEA FILE=HCAPLUS ABB=ON  L6 AND KIT?
L9             9 SEA FILE=HCAPLUS ABB=ON  L7 OR L8
L10            9 SEA FILE=HCAPLUS ABB=ON  L7 OR L8 OR L9
L11           15 SEA L10
L12           13 DUP REMOV L11 (2 DUPLICATES REMOVED)

=> d ibib abs l12 1-13

L12  ANSWER 1 OF 13  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
ACCESSION NUMBER:   2004282234  EMBASE
TITLE:              Detection of IgG antibody to Toxoplasma gondii with rSAG1
                    for immunodiagnosis of toxoplasmosis.
AUTHOR:             Si J.; Tang J.-M.; Xu M.; Yang X.-H.; Yin X.-R.; Cao G.-Q.;
                    Liang Y.-S.; Dai J.-R.; Zhu Y.-C.
CORPORATE SOURCE:   J. Si, Jiangsu Inst. of Parasitic Diseases, Wuxi 214064,
                    China. sijin2001@sohu.com
SOURCE:             Chinese Journal of Microbiology and Immunology, (2004) 24/3
                    (245-248).
                    Refs: 10
                    ISSN: 0254-5101  CODEN: ZWMZDP
COUNTRY:            China
DOCUMENT TYPE:      Journal; Article
FILE SEGMENT:       004     Microbiology
                    005     General Pathology and Pathological Anatomy
                    026     Immunology, Serology and Transplantation
LANGUAGE:           Chinese
SUMMARY LANGUAGE:   English; Chinese
AB   Objective: To establish a rSAG1-ELISA method with purified rSAG1
     fusion protein for immunodiagnosis of toxoplasmosis.
     Methods: rSAG1 fusion protein was expressed and
     purified by Ni(2+) chelating Hi-Trap HP
     column. ELISA plate was coated with different concentrations of
     rSAG1, reacted with pooled positive and negative sera, Goat anti-human IgG
     conjugated to horseradish peroxidase was used as a second antibody. The
     appropriate detecting condition of rSAG1-ELISA assay was determined by
     orthogonal experiment, the reproducibility, sensitivity and specificity of
     the assay were tested. Fifty-two IgG-positive and 40 IgG-negative human
     sera detected with imported IgG-ELISA kit were tested with
     rSAG1-ELISA. Results: The purity of rSAG1 was above 90%; the appropriate
     detecting condition of rSAG1-ELISA was that the concentration of coated
     rSAG1 must be 5μg/ml, the human serum should be 1: 100 diluted, and
     goat anti-human IgG conjugated to horseradish peroxidase was in 1: 2000
     dilution; the CV value for IgG-positive and IgG-negative human sera were
     10.9% and 10.7% respectively; the inhibition rate of the assay was 62.6%;
     the total correspondence rate of rSAG1-ELISA was 87% with imported
     IgG-ELISA kit. The positive correspondence rate and negative
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correspondence rate of rSAG1-ELISA was 81% (42/52) and 95% (38/40) respectively as compared with imported IgG-ELISA **kit** of toxoplasmosis. Conclusion: The rSAG1-ELISA has high sensitivity and specificity and good correspondence rate with imported IgG-ELISA **kit** of toxoplasmosis. rSAG1-ELISA has potential value for immunodiagnosis of toxoplasmosis.

L12 ANSWER 2 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002148415 EMBASE
TITLE: A study of recombinant protective H. pylori antigens.
AUTHOR: Jiang Z.; Tao X.-H.; Huang A.-L.; Wang P.-L.
CORPORATE SOURCE: Dr. Z. Jiang, Department of Gastroenterology, First Affiliated Hospital, Chongqing Univ. of Medical Sciences, Chongqing 400016, China. jzh053@mail.china.com
SOURCE: World Journal of Gastroenterology, (2002) 8/2 (308-311). Refs: 32
ISSN: 1007-9327 CODEN: WJGAF2
COUNTRY: China
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
030 Pharmacology
025 Hematology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Aim: To construct a recombinant vector which can express M(r) 26 000 outer membrane protein (OMP) from Helicobacter pylori (Hp), and to obtain the vaccine protecting against Hp infection and a diagnostic reagent **kit** quickly detecting Hp infection. Methods: The gene encoding the structural M(r) 26 000 outer membrane protein of Hp was amplified from Hp chromosomal DNA by PCR, and inserted in the prokaryotic expression vector pET32a (+), which was transformed into the Top10 E. coli strain. Recombinant vector was selected, identified and transformed into BL-21 (DE3) E. coli strain. The recombinant **fusion** proteins were expressed. The antigenicity of recombinant protein was studied by ELISA or immunoblotting and immunized Balb/c mice. Results: The gene of M(r) 26 000 OMP was amplified to be 594 base pairs, 1.1% of the cloned genes was mutated and 1.51% of amino acid residues was changed, but there was homogeneity between them. The recombinant **fusion** protein encoded objective polypeptides of 198 amino acid residues, corresponding to calculated molecular masses of M(r) 26 000. The level of soluble expression products was about 38.96% of the total cell **protein.** After **purification** by **Ni**-NTA agarose **resin columniation**, the purity of objective protein became about 90%. The ELISA results showed that recombinant **fusion** protein could be recognized by patient serum infected with Hp and rabbit serum immunized with the recombinant protein. Furthermore, Balb/ c mice immunized with the recombinant protein were protected against H. pylori infection. Conclusion: Mr 26 000 OMP may be a candidate vaccine preventing Hp infection.

L12 ANSWER 3 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2002:502155 BIOSIS
DOCUMENT NUMBER: PREV200200502155
TITLE: A comparative study of the human T-cell leukemia virus type 2 integrase expressed in and purified from Escherichia coli and Pichia pastoris.

AUTHOR(S): Piefer, Andrew J.; Jonsson, Colleen B. [Reprint author]
CORPORATE SOURCE: Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM, 88003, USA
cjonsson@nmsu.edu
SOURCE: Protein Expression and Purification, (July, 2002) Vol. 25, No. 2, pp. 291-299. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Sep 2002
Last Updated on STN: 25 Sep 2002

AB The human T-cell leukemia virus type-2 (HTLV-2) integrase (IN) catalyzes the insertion of the viral genome into the host chromosome. HTLV-2 IN was expressed as an N-terminal hexa-histidine tagged protein in the methylotrophic yeast Pichia pastoris and as a C-terminal hexa-histidine **fusion** in Escherichia coli. Maximal IN expression was observed at 48 h post-induction for the yeast system and 2 h post-induction for E. coli. Effective purification strategies were developed using non-ionic and zwitterionic detergents for initial **protein extraction**, followed by a one-step **nickel-chelating chromatography** purification. IN from both sources was routinely greater than 90% pure with yields exceeding 1.5 mg of purified IN per liter of culture for P. pastoris. The relative pI was defined for both INs, pH 5.0-5.4, by 2D-gel electrophoresis. Specific activities for IN purified from E. coli and P. pastoris were calculated from in vitro 3' processing assays and were comparable. In vitro IN assays were also performed to optimize reaction **buffer** pH and metal concentrations for both 3' processing and strand transfer assays. Strand transfer was optimal from pH 6.2-6.8, more than 1.5 pH units below the optimal 3' processing pH of 8.3. IN from both sources showed no enhancement in activity with MnCl2 concentrations greater than 5 mM. The specific activity of P. pastoris purified IN was 0.35 product (pmol)/h/mug IN, and E. coli produced IN was 0.48 product (pmol)/h/mug IN.

L12 ANSWER 4 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2002045382 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11755059
TITLE: Expression of ecdysteroid receptor and ultraspiracle from **Chironomus** tentans (Insecta, Diptera) in E. coli and purification in a functional state.
AUTHOR: Grebe Marco; Spindler-Barth Margarethe
CORPORATE SOURCE: Abteilung fur Allgemeine Zoologie und Endokrinologie, Universitat Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany.
SOURCE: Insect biochemistry and molecular biology, (2002 Feb) 32 (2) 167-74.
Journal code: 9207282. ISSN: 0965-1748.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020124
Last Updated on STN: 20020313
Entered Medline: 20020312

AB Full length clones of ecdysteroid receptor (EcR) and Ultraspiracle (USP) from **Chironomus** tentans were expressed as GST **fusion proteins** in E. coli and **purified** by affinity **chromatography**. The absence of detergents during the purification procedure is essential for retaining receptor function, especially ligand

binding. Presence of USP is mandatory for ligand binding to EcR, but no other cofactors or posttranslational modifications seem to be important, since **Scatchard** plots revealed the same characteristics (two high affinity binding sites for Ponasterone A with K(D1)=0.24+/-0.1nM and K(D2)=3.9+/-1.3.nM) as found in 0.4 M NaCl extracts of **Chironomus** cells. Gel mobility shift assays showed binding of the heterodimer to PAL and DR5 even after removal of the GST-tag, whereas EcR binding to PAL1 is GST-dependent. USP binds preferentially to DR5. Addition of unprogrammed reticulocyte lysate improves ligand binding only slightly. Removal of GST has no effect on (3)H-ponasterone A binding, but alters DNA binding characteristics. Calculation of specific binding (5.3+3.0 nmol/mg GST EcR) revealed that 47+/-26% of **purified** receptor **protein** was able to bind ligand. The addition of purified EcR to cell extracts of hormone resistant subclones of the epithelial cell line from C. tentans, which have lost their ability to bind ligand, restores specific binding of (3)H-ponasterone A.

L12 ANSWER 5 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2002019721 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11437613
TITLE: SwellGel: an affinity **chromatography** technology for high-capacity and high-throughput **purification** of recombinant-tagged **proteins.**
AUTHOR: Draveling C; Ren L; Haney P; Zeisse D; Qoronfleh M W
CORPORATE SOURCE: Perbio Sciences, 2202 N. Bartlett Avenue, Milwaukee, Wisconsin, 53202-1009, USA.
SOURCE: Protein expression and purification, (2001 Jul) 22 (2) 359-66.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011207

AB The revolution in genomics and proteomics is having a profound impact on drug discovery. Today's protein scientist demands a faster, easier, more reliable way to **purify proteins.** A high capacity, high-throughput new technology has been developed in Perbio Sciences for affinity **protein purification.** This technology utilizes selected **chromatography** media that are dehydrated to form uniform aggregates. The SwellGel aggregates will instantly rehydrate upon addition of the **protein** sample, allowing **purification** and direct performance of multiple assays in a variety of formats. SwellGel technology has greater stability and is easier to handle than standard wet **chromatography resins** . The microplate format of this technology provides high-capacity, high-throughput features, recovering milligram quantities of protein suitable for high-throughput screening or biophysical/structural studies. Data will be presented applying SwellGel technology to recombinant 6x His-tagged protein and glutathione-S-transferase (GST) **fusion protein purification.**

L12 ANSWER 6 OF 13 BIOSIS on STN
ACCESSION NUMBER: 2001:378784 BIOSIS
DOCUMENT NUMBER: PREV200100378784

TITLE: Affinity of the monoclonal antibody M1 directed against the FLAG peptide.
AUTHOR(S): Einhauer, A.; Jungbauer, A. [Reprint author]
CORPORATE SOURCE: Institute of Applied Microbiology, University of Agricultural Sciences, Muthgasse 18, A-1190, Vienna, Austria
jungbaue@hp01.boku.ac.at
SOURCE: Journal of Chromatography A, (29 June, 2001) Vol. 921, No. 1, pp. 25-30. print.
CODEN: JOCRAM. ISSN: 0021-9673.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The FLAG (Sigma, St. Louis, MO, USA) peptide is a frequently used hydrophilic and immunogenic **fusion** tag which was specifically designed to facilitate rapid purification by immunoaffinity **chromatography.** The monoclonal antibody M1 recognizes the free N-terminus of the peptide tag in a **calcium** dependent manner. Dissociation of the complex can be performed by the addition of **chelating** agents such as EDTA. This effect can be exploited for immunoaffinity **purification** of FLAG-tagged **fusion proteins.** Kinetic information obtained from monitoring interactions in real-time measurement (Biacore 2000) using surface plasmon resonance as detection principle did not show any difference for association and dissociation rate constants in the presence (ka = 3.03cntdot103 M-1 s-1 kd = 1.25cntdot10-3 s-1) and in the absence of Ca2+ (ka = 3.59cntdot103 M-1 s-1, kd = 1.16cntdot10-3 s-1). These findings corroborate the reports from Mol. Immunol. 33 (1996) 601-608 describing similar binding analyzed by enzyme-linked immunosorbent assay experiments. These investigations are in contrast to the observations in immunoaffinity **chromatography** with immobilized anti-FLAG antibody M1.

L12 ANSWER 7 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:233629 BIOSIS
DOCUMENT NUMBER: PREV200000233629
TITLE: Expression in Escherichia coli and simple **purification** of human Fhit **protein.**
AUTHOR(S): Pawelczyk, Tadeusz [Reprint author]; Kowara, Renata; Golebiowski, Filip; Matecki, Andrzej
CORPORATE SOURCE: Department of Molecular Medicine, Medical University of Gdansk, 80-211, Gdansk, Poland
SOURCE: Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 320-326. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: Genbank-U46922
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB The fragile histidine triad (Fhit) protein is a homodimeric protein with diadenosine 5',5'''-P1,P3-triphosphate (Ap3A) asymmetrical hydrolase activity. We have cloned the human cDNA Fhit in the pPROEX-1 vector and expressed with high yield in Escherichia coli with the sequence Met-Gly-His6-Asp-Tyr-Asp-Ile-Pro-Thr-Thr followed by a rTEV protease cleavage site, denoted as "H6TV," **fused** to the N-terminus of Fhit. Expression of H6TV-Fhit in BL21(DE3) cells for 3 h at 37degreeC produced 30 mg of H6TV-Fhit from 1 L of cell culture (apprx4 g of cells). The H6TV-Fhit **protein** was **purified** to homogeneity in a

single step, with a yield of 80%, using **nickel**-nitrilotriacetate **resin** and imidazole **buffer** as eluting agent. Incubation of H6TV-Fhit with rTEV protease at 4degreeC for 24 h resulted in complete cleavage of the H6TV peptide. There were no unspecific cleavage products. The **purified** Fhit **protein** could be stored for 3 weeks at 4degreeC without loss of activity. The pure protein was stable at -20degreeC for at least 18 months when stored in **buffer** containing 25% glycerol. Purified Fhit was highly active, with a Km value for Ap3A of 0.9 muM and a kcat(monomer) value of 7.2 +- 1.6 s-1 (n = 5). The catalytic properties of unconjugated Fhit protein and the H6TV-Fhit **fusion** protein were essentially identical. This indicates that the 24-amino-acid peptide containing the six histidines **fused** to the N-terminus of Fhit does not interfere in forming the active homodimers or in the binding of Ap3A.

L12 ANSWER 8 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:233628 BIOSIS
DOCUMENT NUMBER: PREV200000233628
TITLE: Expression of human cardiac-specific homeobox protein in Escherichia coli.
AUTHOR(S): Zhao, Jing-Hui; Xu, Zhao; Hua, Zi-Chun [Reprint author]
CORPORATE SOURCE: State Key Laboratory of Pharmaceutical Biotechnology, Department of Biochemistry, School of Life Sciences, Nanjing University, Nanjing, 210093, China
SOURCE: Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 316-319. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB Human cardiac-specific homeobox protein cDNA (hCsx) was cloned into expression plasmid pET32a and **fused** with Escherichia coli thioredoxin (Trx). The Trx-Csx **fusion** protein was under the control of bacteriophage T7 promoter. When expressed in E. coli BL21(DE3), about half of the recombinant Trx-Csx products existed in the form of insoluble inclusion bodies. When coexpressed with human protein disulfide isomerase, more than 90% of Trx-Csx products accumulated in the soluble form in the cell lysate. The recombinant Csx **fusion** **protein** was **purified** by one-step metal-**chelating** affinity **chromatography**.

L12 ANSWER 9 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:220905 BIOSIS
DOCUMENT NUMBER: PREV200000220905
TITLE: **Purification** of His-tagged **proteins** by immobilized **chelate** affinity **chromatography**: The benefits from the use of organic solvent.
AUTHOR(S): Franken, Kees L. M. C. [Reprint author]; Hiemstra, Hoebert S. [Reprint author]; van Meijgaarden, Krista E. [Reprint author]; Subronto, Yanri [Reprint author]; den Hartigh, J.; Ottenhoff, Tom H. M. [Reprint author]; Drijfhout, Jan W. [Reprint author]
CORPORATE SOURCE: Department of Immunohematology and Blood Bank, Leiden University Medical Center, 2300 RC, Leiden, Netherlands
SOURCE: Protein Expression and Purification, (Feb., 2000) Vol. 18, No. 1, pp. 95-99. print.

CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 May 2000
Last Updated on STN: 5 Jan 2002

AB Recombinant **proteins** overexpressed in and **purified** from Escherichia coli contain impurities that are toxic in biological assays. The application of affinity purification procedures is often not sufficient to remove these toxic components. We here describe a simple and fast, one-step protocol to remove these impurities highly efficiently. Four recombinant proteins were overexpressed in E. coli as His-tagged **fusion proteins** and **purified** by immobilized metal **chelate** affinity **chromatography** on **Ni** -NTA beads. Depending on the protein, the composition of the lysis **buffer**, and the washing protocol, various impurities appeared to be present in the **purified protein** preparations. Here we show how the use of 60% isopropanol during washing steps removed most of these contaminants from the end products. In addition to the removal of proteins that aspecifically adhere to the beads or to the tagged protein, this procedure was particularly useful in removing endotoxins. Moreover, we show that detergents such as NP-40, that are necessarily employed during lysis, are also efficiently removed. Finally, we show that proteins are able to refold correctly after isopropanol treatment. Thus, the resulting end products contain significantly less contaminating E. coli proteins, endotoxins, and detergents.

L12 ANSWER 10 OF 13 MEDLINE on STN
ACCESSION NUMBER: 1999356759 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10427752
TITLE: Selective adsorption of poly-His tagged glutaryl acylase on tailor-made metal **chelate** supports.
AUTHOR: Armisen P; Mateo C; Cortes E; Barredo J L; Salto F; Diez B; Rodes L; Garcia J L; Fernandez-Lafuente R; Guisan J M
CORPORATE SOURCE: Hispanagar S.A., Burgos, Spain.
SOURCE: Journal of chromatography. A, (1999 Jul 2) 848 (1-2) 61-70. Journal code: 9318488.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990913
Last Updated on STN: 20020420
Entered Medline: 19990830

AB A poly-His tag was **fused** in the glutaryl acylase (GA) from Acinetobacter sp. strain YS114 cloned in E. coli yielding a fully active enzyme. Biochemical analyses showed that the tag did not alter the maturation of the chimeric GA (poly-His GA) that undergoes a complex post-translational processing from an inactive monomeric precursor to the active heterodimeric enzyme. This enzyme has been used as a model to develop a novel and very simple procedure for one-step **purification** of poly-His **proteins** via immobilized metal-ion affinity **chromatography** on tailor-made supports. It was intended to improve the selectivity of adsorption of the target protein on tailor-made **chelate** supports instead of performing a selective desorption. The rate and extent of the adsorption of **proteins** from a crude **extract** from E. coli and of pure poly-His tagged GA on different metal **chelate** supports was studied. Up to 90% of proteins from E. coli were adsorbed on commercial **chelate** supports having a high density of ligands attached to the

support through long spacer arms, while this adsorption becomes almost negligible when using low ligand densities, short spacer arms and Zn2+ or Co2+ as cations. On the contrary, poly-His GA adsorbs strongly enough on all supports. A strong affinity interaction between the poly-His tail and a single **chelate** moiety seems to be the responsible for the adsorption of poly-His GA. By contrast, multipoint weak interactions involving a number of **chelate** moieties seem to be mainly responsible for adsorption of natural proteins. By using tailor-made affinity supports, a very simple procedure for one-step purification of GA with minimal adsorption of host proteins could be performed. Up to 20 mg of GA were adsorbed on each ml of **chelate** support while most of accompanying proteins were **hardly** adsorbed on such supports. Following few washing steps, the target enzyme was finally recovered (80% yield) by elution with 50 mM imidazole with a very high increment of specific activity (up to a 120 purification factor).

L12 ANSWER 11 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 97095716 EMBASE
DOCUMENT NUMBER: 1997095716
TITLE: Folding-dependent binding of thyrotropin (TSH) and TSH receptor autoantibodies to the murine TSH receptor ectodomain.
AUTHOR: Vlase H.; Matsuoka N.; Graves P.N.; Magnusson R.P.; Davies T.F.
CORPORATE SOURCE: Dr. T.F. Davies, Mount Sinai Medical Center, Box 1055, 1 Gustave L. Levy Place, New York, NY 10029, United States
SOURCE: Endocrinology, (1997) 138/4 (1658-1666).
Refs: 38
ISSN: 0013-7227 CODEN: ENDOAO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The mouse TSH receptor ectodomain (mTSHR-ecd)was amplified from murine thyroid complementary DNA and ligated into the pAcGP67B insect cell vector, and the nucleotide sequence was confirmed. Employing a baculovirus-insect cell system, the mTSHR-ecd (amino acids 22-415) was expressed as a **fusion** protein with the gp67 insect cell signal sequence at the NH2-terminus and a C-terminal six-histidine tag. Protein expression was assessed by Western blot using a murine monoclonal antibody (recognizing amino acids 22-35) and a rabbit antipeptide antibody (recognizing amino acids 397-415). These antibodies detected two principal species of mTSHR-ecd, one glycosylated (66 kDa) and one nonglycosylated (52 kDa), in cell lysates of infected insect cells. More than 10% of these species were present in a water-soluble (cytosolic) fraction. This fraction was then used to purify, under native conditions, 100-μg amounts of mTSHR-ecd using **nickel**-nitrilo-triacetic (**Ni** - NTA/**resin chromatography.** The purified cytosolic mTSHR-ecd migrated as a homogeneous 66-kDa band visible on Coomassie blue-stained gels and was confirmed by Western blotting. We also purified the mTSHR-ecd from total cell lysates under denaturing conditions, followed by 'in vitro' refolding on the **Ni**-NTA **column.** Under these conditions, milligram amounts of soluble mT-SHR- ecd were obtained. This material consisted primarily of the 66-kDa glycosylated form, but in addition contained four or five lower molecular mass, partially glycosylated **intermediates** and the 52-kDa nonglycosylated form. Deglycosylation with either endoglycosidase F or H,

reduced all mTSHR- ecd glycosylated species to a 52-kDa non-glycosylated form. Both the cytosolic and refolded mTSHR-ecd preparations inhibited the binding of [125]TSH to the full-length human TSHR expressed in Chinese hamster ovary cells in a dose-dependent manner, with similar affinities. The affinity of such interactions was 3 orders of magnitude less than observed with native porcine TSHR and was further reduced by unfolding the mTSHR-ecd preparations. The cytosolic and refolded mTSHR-ecd were also recognized by hT-SHR autoantibodies in the serum of patients with hyperthyroid Graves' disease. Such autoantibody binding to mTSHR-ecd was also markedly reduced by unfolding the antigen. These results demonstrated the successful production of large quantities of well characterized, biologically active, mTSHR-ecd antigen. In addition, the data showed that although the ectodomain of the mTSHR bound TSH, intact holoreceptor may be required for high affinity ligand binding. Whether the transmembrane region is required for direct ligand binding, as seen for other G protein-linked receptors, or whether it is needed to stabilize the ligand binding to the ectodomain and maintain a correctly folded state, remains unclear.

L12 ANSWER 12 OF 13 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 97075938 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8918261
TITLE: A multifunctional vector system for heterologous expression of proteins in Escherichia coli. Expression of native and hexahistidyl **fusion proteins**, rapid **purification** of the **fusion proteins**, and removal of **fusion** peptide by Kex2 protease.
AUTHOR: Ghosh S; Lowenstein J M
CORPORATE SOURCE: Biochemistry Department, Brandeis University, Waltham, MA 02254, USA.
SOURCE: Gene, (1996 Oct 17) 176 (1-2) 249-55.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19970107

AB Vectors have been constructed for the general purpose of expressing foreign proteins in E. coli. These vectors allow the production in high yield of either native proteins or of **fusion** proteins which contain, at their amino terminus, the peptide Met Gly His6 Ser Gly Leu Phe Lys Arg/, where Leu Phe Lys Arg/ is the recognition site for Kex2 protease which cleaves at the site indicated by /. The His6 sequence is used as a ligand for the one-step affinity **purification** of the expressed **proteins** on **columns** containing **Ni** or **Zn** ions **chelated** to iminodiacetic acid-agarose. After affinity **chromatography**, the **purification peptide** is cleaved off with Kex2 protease from Saccharomyces cerevisiae. The vectors also allow site-directed mutagenesis and sequencing of the cloned gene to be expressed without any **intermediate** subcloning. For practical examples of over-expression, affinity purification, and removal of the **purification peptide**, we chose a high-molecular-weight protein, phospholipase C gamma 1 (PLC gamma 1, M(r) 148,000) and a low-molecular-weight protein, Hit-1 (M(r) 16,000). Both were obtained pure and in high yield. PLC gamma 1 was fully active; the function of

Hit-1 is not known. A set of companion vectors for **co** -expression of additional proteins has also been developed. These allow expression of proteins which enhance the production or activity of the protein of primary interest and of proteins which exhibit trans-interactions.

L12 ANSWER 13 OF 13 JICST-EPlus COPYRIGHT 2005 JST on STN
ACCESSION NUMBER: 960902029 JICST-EPlus
TITLE: Preparation of the DHFR **Fusion** Protein of the Antigen Peptide for Human TIMP-1.
AUTHOR: IMAMURA TAIZO; SHIBATA YASUKO; TAKIGUCHI HISASHI
CORPORATE SOURCE: Nihon Univ., Sch. of Dent. at Matsudo
SOURCE: Nichidai Koku Kagaku (Nihon University Journal of Oral Science), (1996) vol. 22, no. 3, pp. 222-232. Journal Code: Z0705A (Fig. 6, Ref. 23)
ISSN: 0385-0145
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New

AB The formation and degradation of extracellular matrix (ECM) components are closely related to the many physiological and pathological processes. The degradation of connective tissues is triggered by the activation of certain matrix metalloproteinase (MMP) during physiological and pathological turnover. This activation process is controlled by enzymatic cascade involved in the ECM metabolism. One additional characteristic of MMP family is that they are inhibited by specific proteinase inhibitors known as the tissue inhibitors of metalloproteinases (TIMPs). Both activated- and preactivated-MMPs are inhibited by TIMPs. These observations have suggested that MMPs activities are tightly regulated by both positive and negative control systems in several tissues. In this study, we have attempted to construct the recombinant **fusion** protein consisting of dihydrofolate reductase (DHFR) and TIMP-1 (rDHFR-TIMP-1 protein) to prepare rabbit IgG against human TIMP-1. To obtain the **fusion** protein, the plasmid pQE40 (the QIA expressionist **kit**) was used. This plasmid is composed of several gene cassettes including a promoter, 6 His, DHFR, and MCS. To obtain the cDNA for TIMP-1, total RNAs from fibroblastic cells of human periodontal ligament were used as a template for RT-PCR. PCR amplified TIMP-1 gene was gel purified, and inserted into the pGEM-3Zf(+) and pQE40. After the confirmation of TIMP-1 cDNA insert by DNA sequencing, DNA fragment spanning from promoter to cDNA insert was further subcloned into the pHSG298 to obtain vast amount of the **fusion** protein following the induction with IPTG. rDHFR-TIMP-1 **fusion** protein was present in the insoluble fraction of transformant. Because the NH2-terminal end of this recombinant **fusion** protein is the 6 His tag amino acid stretch, **purification** of this **protein** was facilitated by passing through onto the **Ni**-NTA **resin column.** (author abst.)

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=> d his ful

     FILE 'REGISTRY' ENTERED AT 14:53:15 ON 04 FEB 2005
L1             7 SEA ABB=ON  (IRON OR CALCIUM OR ALUMINUM OR NICKEL OR ZINC OR
                  COBALT OR COPPER)/CN

     FILE 'HCAPLUS' ENTERED AT 14:53:50 ON 04 FEB 2005
L2        111827 SEA ABB=ON  (?PEPTID? OR ?PROTEIN?)(4A)(?PURIF? OR ?WASH? OR
                  ?EXTRACT? OR ?ELUT?)
L3          3656 SEA ABB=ON  L2 AND (?CHELAT? OR ?RESIN?)
L4          1288 SEA ABB=ON  L3 AND (L1 OR ?IRON? OR FE OR ?CALCIUM? OR CA OR
                  ?ALUMINUM? OR AL OR ?NICKEL? OR NI OR ?ZINC? OR ZN OR ?COBALT?
                  OR CO OR ?COPPER? OR CU)
L5           229 SEA ABB=ON  L4 AND (?FUSE? OR ?FUSION?)
L6           177 SEA ABB=ON  L5 AND (?CHROMATOG? OR ?BUFFER? OR ?COLUMN?)
L7             4 SEA ABB=ON  L6 AND (?HARD? OR ?INTERMED?)
L8             6 SEA ABB=ON  L6 AND KIT?
L9             9 SEA ABB=ON  L7 OR L8
L10            9 SEA ABB=ON  L7 OR L8 OR L9

     FILE 'MEDLINE, BIOSIS, EMBASE, JAPIO, JICST-EPLUS, COMPENDEX' ENTERED AT
     14:57:27 ON 04 FEB 2005
L11           15 SEA ABB=ON  L10
L12           13 DUP REMOV L11 (2 DUPLICATES REMOVED)
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9 cits from CA Plus

13 cits from other databases

Inventor Search

=> d ibib abs ind l3 1-3

L3 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:759732 HCAPLUS
DOCUMENT NUMBER: 141:273989
TITLE: Purification of fusion proteins using immobilized bi-metal affinity chromatography
INVENTOR(S): **Tchaga, Grigoriy S.; Jokhadze, George G.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp., Cont.-in-part of U.S. Pat. Appl. 2002 164,718.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
US 2004180415	A1	20040916	US 2004-762588		20040121
US 2002164718	A1	20021107	US 2001-858332		20010515
PRIORITY APPLN. INFO.:			US 2001-858332	A2	20010515
			US 2003-441804P	P	20030121
			US 1998-101867P	P	19980925
			US 1999-404017	B2	19990923

AB The present invention relates to IMAC (Immobilized Metal Affinity Chromatog.). The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first metal ion chelate resin having a first immobilized metal ion; eluting any bound protein from the first metal ion chelate resin, to produce an eluate; contacting the eluate with a second metal ion chelate resin having a second immobilized metal ion; and eluting any bound protein from the second metal ion chelate resin. Also provided are kits for use in practicing the subject methods. An illustrative purification protocol for Bi-MAC (Bi-Metal Affinity Chromatog.) is shown. The subject methods find use in a variety of protein purification applications.

IC ICM C12N009-00
NCL 435183000; 530412000
CC 9-3 (Biochemical Methods)
ST fusion protein purifn bimetal affinity chromatog IMAC; metal ion chelating resin affinity peptide fusion protein purifn
IT Affinity chromatography
(IMAC; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Washing
(buffer for; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Extractants
(buffer; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Denaturants
(denaturing conditions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Buffers
(extraction, wash and elution; purification of fusion proteins using immobilized

bi-metal affinity chromatog.)
IT Cations
(hard and intermediate metal ions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Affinity chromatographic stationary phases
(metal ion affinity peptide; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Peptides, uses
RL: NUU (Other use, unclassified); USES (Uses)
(metal ion affinity peptide; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Immobilization, molecular or cellular
(of metal ions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Liquid chromatographic columns
Test kits
(purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT Fusion proteins (chimeric proteins)
RL: PUR (Purification or recovery); PREP (Preparation)
(purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT 7429-90-5, Aluminum, uses 7439-89-6, Iron, uses 7440-70-2, Calcium, uses 14127-61-8, Calcium 2+, uses 20074-52-6, Iron 3+, uses 22537-23-1, Aluminum 3+, uses
RL: NUU (Other use, unclassified); USES (Uses)
(immobilized, hard metal ion; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT 7440-02-0, Nickel, uses 7440-48-4, Cobalt, uses 7440-50-8, Copper, uses 7440-66-6, Zinc, uses 14701-22-5, Nickel 2+, uses 15158-11-9, Copper 2+, uses 22541-53-3, Cobalt 2+, uses 23713-49-7, Zinc 2+, uses
RL: NUU (Other use, unclassified); USES (Uses)
(immobilized, intermediate metal ion; purification of fusion proteins using immobilized bi-metal affinity chromatog.)
IT 54017-28-6 85139-13-5 91859-00-6 92000-72-1 98849-88-8 158734-08-8 444311-97-1 474426-21-6 474426-23-8 474426-25-0 474426-27-2 474426-29-4
RL: PRP (Properties)
(unclaimed sequence; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

L3 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:850241 HCAPLUS
DOCUMENT NUMBER: 137:365958
TITLE: Metal ion affinity peptides and methods for using the same in protein purification methods
INVENTOR(S): **Tchaga, Grigoriy S.; Jokhadze, George G.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 23 pp., Cont.-in-part of U. S. Ser. No. 404,017, abandoned.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002164718	A1	20021107	US 2001-858332	20010515

US 2004180415 A1 20040916 US 2004-762588 20040121
PRIORITY APPLN. INFO.: US 1998-101867P P 19980925
US 1999-404017 B2 19990923
US 2001-858332 A2 20010515
US 2003-441804P P 20030121
OTHER SOURCE(S): MARPAT 137:365958

AB The present invention provides metal ion affinity peptides, fusion proteins comprising metal ion affinity peptides, and polynucleotides encoding the fusion proteins. The invention further provides recombinant vectors comprising subject polynucleotides, and host cells comprising the recombinant vectors. The invention further provides methods and kits for purifying a fusion protein comprising a metal ion affinity peptide.

IC ICM C12P021-02
ICS C12N005-06; C12P021-04; C07K014-435; C07K007-08
NCL 435069700
CC 9-3 (Biochemical Methods)
Section cross-reference(s): 3, 6
ST metal ion affinity chromatog genetic recombination fusion protein purifn
IT Peptides, uses
RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)
(affinity; metal ion affinity peptides and methods for using the same in protein purification methods)
IT Chromatographs
(columns, purification kit; metal ion affinity peptides and methods for using the same in protein purification methods)
IT Buffers
(extraction, washing, elution; metal ion affinity peptides and methods for using the same in protein purification methods)
IT Prokaryota
(host cell; metal ion affinity peptides and methods for using the same in protein purification methods)
IT Affinity chromatography
Genetic vectors
Immobilization, molecular or cellular
Purification
Recombination, genetic
Washing
(metal ion affinity peptides and methods for using the same in protein purification methods)
IT Metals, uses
RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)
(metal ion affinity peptides and methods for using the same in protein purification methods)
IT Proteins
RL: PRP (Properties); PUR (Purification or recovery); PREP (Preparation)
(metal ion affinity peptides and methods for using the same in protein purification methods)
IT Fusion proteins (chimeric proteins)
RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)
(peptide fused to metal ion affinity peptide; metal ion affinity peptides and methods for using the same in protein purification methods)
IT Polynucleotides
RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)
(recombinant; metal ion affinity peptides and methods for using the same in protein purification methods)
IT 474426-21-6 474426-23-8 474426-25-0 474426-27-2 474426-29-4

RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)
(amino acid sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

IT 14127-61-8, Ca2+, uses 14701-22-5, Ni2+, uses 15158-11-9, Cu2+, uses 20074-52-6, Fe3+, uses 22537-23-1, Al3+, uses 22541-53-3, Co2+, uses 23713-49-7, Zn2+, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(metal ion affinity peptides and methods for using the same in protein purification methods)

IT 475131-38-5 475131-39-6 475131-40-9 475131-41-0 475131-42-1 475131-43-2
RL: PRP (Properties)
(unclaimed nucleotide sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

IT 92000-72-1 98849-88-8 444311-97-1 475084-76-5 475084-78-7 475131-35-2 475131-37-4
RL: PRP (Properties)
(unclaimed sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

L3 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:736412 HCAPLUS
DOCUMENT NUMBER: 131:348793
TITLE: Compositions and methods for protein purification based on a metal ion affinity site
INVENTOR(S): **Tchaga, Grigoriy; Jokhadze, George G.**
PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9957992	A1	19991118	WO 1999-US10662		19990514
W: AU, CA, JP					
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
AU 9941877	A1	19991129	AU 1999-41877		19990514
PRIORITY APPLN. INFO.:			US 1998-78687	A	19980514
			WO 1999-US10662	W	19990514

AB The present invention describes introducing metal ion affinity sequences into recombinant proteins to allow for purification and/or immobilization of these proteins. The present invention provides a fusion protein, comprising: a protein of interest fused at its amino-terminus or carboxy-terminus to at least one affinity peptide, said fusion protein having a formula R1-(HXn)m-R2, wherein R1 or R2 is said protein of interest, n = 1-8, m = 2-30, and wherein if n = 1 for more than two adjacent units of HX, at least one X must be asparagine, phenylalanine, tryptophan, tyrosine, lysine, methionine, arginine, glutamine, or cysteine.

IC ICM A23J001-00
ICS C12N015-63; C12N015-00; C12N001-21; C12P021-04; A61K038-00; C07K001-00; C07H021-04

CC 9-16 (Biochemical Methods)

ST compn protein purifn metal ion affinity

IT Peptides, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Affinity; compns. and methods for protein purification based on a metal ion affinity site)
IT Affinity
Bond cleavage
Cations
Chelating agents
Composition
DNA sequences
Extraction
Immobilization, biochemical
Muscle
Purification
(compns. and methods for protein purification based on a metal ion affinity site)
IT Fusion proteins (chimeric proteins)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(compns. and methods for protein purification based on a metal ion affinity site)
IT Proteins, general, preparation
RL: BPN (Biosynthetic preparation); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)
(compns. and methods for protein purification based on a metal ion affinity site)
IT Amino acids, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(compns. and methods for protein purification based on a metal ion affinity site)
IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(green fluorescent, UV mutant; compns. and methods for protein purification based on a metal ion affinity site)
IT Genetic vectors
(recombinant; compns. and methods for protein purification based on a metal ion affinity site)
IT 52-90-4, Cysteine, biological studies 56-85-9, Glutamine, biological studies 56-87-1, Lysine, biological studies 60-18-4, Tyrosine, biological studies 63-68-3, Methionine, biological studies 63-91-2, Phenylalanine, biological studies 70-47-3, Asparagine, biological studies 71-00-1, Histidine, biological studies 73-22-3, Tryptophan, biological studies 74-79-3, L-Arginine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(compns. and methods for protein purification based on a metal ion affinity site)
IT 250266-36-5P
RL: BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)
(compns. and methods for protein purification based on a metal ion affinity site)
IT 9001-92-7, Protease
RL: CAT (Catalyst use); USES (Uses)
(compns. and methods for protein purification based on a metal ion affinity site)
IT 14701-22-5, uses
RL: NUU (Other use, unclassified); PEP (Physical, engineering or chemical

process); PROC (Process); USES (Uses)
(compns. and methods for protein purification based on a metal ion affinity site)

IT 9001-60-9P, Lactate dehydrogenase
RL: PUR (Purification or recovery); PREP (Preparation)
(compns. and methods for protein purification based on a metal ion affinity site)

IT 250673-92-8P
RL: BPN (Biosynthetic preparation); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)
(nucleotide sequence; compns. and methods for protein purification based on a metal ion affinity site)

IT 250676-83-6, 3: PN: WO9957992 SEQID: 3 unclaimed DNA 250676-84-7, 4: PN: WO9957992 SEQID: 4 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; compns. and methods for protein purification based on a metal ion affinity site)

IT 250662-75-0 250676-82-5
RL: PRP (Properties)
(unclaimed protein sequence; compns. and methods for protein purification based on a metal ion affinity site)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT